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DEPARTAMENTO DE CIÊNCIAS DA VIDA

FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE DE COIMBRA

Population dynamics and productivity of *Echinogammarus marinus* (Leach) in the Mondego estuary throughout three decades

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Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia, realizada sob a orientação científica do Professor Doutor João Carlos Marques (Universidade de Coimbra) e da Doutora Irene Martins (Universidade de Coimbra)

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RESUMO

Anfípodes são pequenos crustáceos com uma distribuição ao longo de todos os habitats marinhos e de água doce. *Echinogammarus marinus* (Amphipoda, Gammaridae) é uma espécie amplamente distribuída ao longo da costa europeia, sendo o estuário do Mondego (costa oeste de Portugal) considerado o limite sul da sua distribuição. Estudos prévios determinaram que *E. marinus* é a espécie mais abundante nos habitats intertidais rochosos, tendo um papel crucial na organização trófica do estuário do Mondego.

A ocorrência de intersexualidade tem sido largamente descrita em crustáceos, incluindo populações de *E. marinus* do norte da Europa e mais recentemente no estuário do Mondego. Dado que esta anomalia traz custos associados, como fecundidade e fertilidade reduzidas, baixo sucesso no emparelhamento, maturação retardada e oscilações na proporção de machos/fêmeas, um dos objectivos deste trabalho foi determinar as possíveis consequências da intersexualidade para as populações de *E. marinus* no estuário do Mondego. Tendo em conta a importância desta espécie para a rede trófica, também foram definidos como objectivos: estudar a dinâmica populacional e produção secundária de *E. marinus* no estuário do Mondego, comparando os resultados com os obtidos em estudos anteriores; determinar os principais factores bióticos e abióticos que contribuíram para a variação espacial e temporal desta espécie. Para tal, foram amostradas mensalmente três populações de *Echinogammarus marinus* (Pranto, Armazéns e Fontela), desde Abril de 2009 até Março de 2010.

A análise estatística dos dados bióticos e abióticos demonstrou que, embora em declínio durante o período de amostragem, as densidades obtidas foram superiores às descritas na década de 90 mas longe das obtidas nos anos 80. Também se verificaram variações espaciais e temporais, com os valores mais altos de densidade a serem

registados para a população da Fontela durante a primavera. De acordo com os tratamentos estatísticos, a salinidade foi o factor com maior preponderância na variação observada. A incidência de intersexualidade foi considerada insignificante (< 0,1%), sendo, por isso, impossível determinar os seus efeitos nas populações de *E. marinus* do estuário do Mondego. Foi ainda observado que a actividade sexual e o recrutamento foram contínuos e que a proporção de machos/fêmeas tendeu essencialmente para os machos, o que está de acordo com os resultados do estudo dos anos 90 mas difere do estudo dos anos 80, onde o recrutamento foi descontínuo e a proporção machos/fêmeas tendeu para as fêmeas. Os valores de produção obtidos também foram superiores aos registados nos anos 90 mas inferiores aos obtidos no estudo dos anos 80.

Tendo em conta os dados obtidos ao longo destas três décadas, o presente estudo contribuiu para avaliação ecológica da evolução das populações de *Echinogammarus marinus* no estuário do Mondego face a diferentes níveis de perturbação ambiental, fornecendo provas evidentes da progressiva recuperação do sistema desde a implementação de medidas de mitigação.

Palavras-chave: *Echinogammarus marinus*, dinâmica populacional, factores ambientais, produtividade

ABSTRACT

Amphipods are small crustaceans ubiquitously distributed in all marine and freshwater habitats. *Echinogammarus marinus* (Amphipoda, Gammaridae) is a widely distributed species throughout the European coast, being the Mondego estuary (west coast of Portugal) its southern limit of distribution. Previous studies have determined that *E. marinus* is the most abundant species in the intertidal rocky habitats, playing a key role in the trophic organization of the Mondego estuary.

Intersexuality incidence has been extensively reported in crustaceans, including northern European populations of *E. marinus* and more recently in the Mondego estuary. Since this condition has fitness costs, namely lower fecundity and fertility, reduced pairing success, delayed maturation and sex-ratio fluctuations, one of the aims of this work was to access its consequences to the *E. marinus* population dynamics and productivity on the Mondego estuary.

Considering the importance of this species to the trophic network, emphasized by the previous studies, we also aimed to study *E. marinus* population dynamics and secondary production on the Mondego estuary, comparing the results with the ones obtained in the previous studies, and to access the main biotic and abiotic variables contributing to this species spatial and temporal variation. To do that, three *Echinogammarus marinus* populations (Pranto, Armazéns and Fontela) were sampled monthly, from April 2009 to March 2010.

The statistical analysis on the biotic and abiotic data showed that, although declining during the study period, density values were higher than the ones reported in the 90's study but still lower than the values from the 80's study. In addition, spatial and temporal variation was observed, with the highest values being observed in Fontela during spring. According to results, salinity and ammonia were the main envrinomental

variables influencing the observed variation. Intersexuality incidence was insignificant (< 0.1%), therefore, we were unable to determine its effects on *E. marinus* populations. Sexual activity and recruitment were continuous, and a male biased sex-ratio was observed, which was in accordance with the 90's study but not with the 80's, where recruitment was discontinuous and females were predominant. Production rates were also higher than in the 90's study but lower than the ones observed in the 80's.

Considering all data throughout the three decades, this work contributed to understand the evolution of the populations of *Echinogammarus marinus* on the Mondego estuary relatively to different levels of environmental preasure, providing solid evidence of the system's recovery since the implementation of mitigation measures.

Keywords: *Echinogammarus marinus*, population dynamics, environmental factors, productivity

CONTENTS

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

1.1 General considerations on Amphipods

Amphipods are small crustaceans that exist in all available marine and freshwater habitats and in some humid terrestrial habitats. They occur in superficial and underground waters, from intertidal zones to abyssal depths, coastal forests to higher altitudes, tropical to polar regions (Marques, 1989). The suborder Gammaridea constitutes the most abundant and diverse group of amphipods, ranging from marine to freshwater areas and even certain moist terrestrial habitats (Reish and Barnard, 1979). Marine Gammaridea are ubiquitously distributed and can be found in all regions, in all habitats and at most depths (Barnard, 1969; Bousefield, 1978).

Organisms of the order Amphipoda have a very important function on the communities of both hard and soft substrates (Marques and Nogueira, 1991), having been considered the most efficient scavengers of sea bottoms and shores, probably clearing up and recycling more organic shore debris than any other animal (Schmitt, 1968). They are also very useful tools to assess environmental quality of aquatic systems due to their affinity for bioaccumulation of contaminants, which consequently makes them vulnerable to chemical pollution (Borgmann *et al.*, 1989).

The order Amphipoda includes organisms capable of almost every trophic regime such as herbivores, predating carnivores, detritivorous and filtering omnivores, thus constituting the main secondary and tertiary producers in aquatic systems and an extremely important link between their energy cycles. Amphipods are also able to convert vegetal and animal proteins in usable forms by larger animals, especially birds and ultimately humans (Bousfield, 1978, 1981).

Morphologically, a “shrimplike” appearance resulting from lateral body compression and sessile compound eyes distinguish amphipods, although some species are blind. A carapace is not present and the first and sometimes the second thoracic segments are fused with the head (Grosse *et al*. 1986). Adult amphipods can range in size from less than 1cm to about 28cm, the largest being an undescribed lysianassid that was photographed in the abyssal Pacific Ocean (Schmidt, 1968).

Female Amphipoda spawn via a mating embrace (amplexus) with males, which can last for hours or days. Following molting and mating, eggs are laid through two ventral pores in the female's sixth thoracic sternite and can number from 1 to 200 or more. Thin, tube-dwelling Gammarids have the fewest eggs, which tend to be large or contain large amounts of yolk. Because of the large size of the eggs, some young females can carry only one, while fully mature females carry three or four. In Gammarids, up to one-half of the eggs may die before hatching and juveniles are generally held in the brood pouch for a few hours to a few days after hatching before they are released (Barnard, 1969; Reish and Barnard, 1979).

The duration of embryonic development in amphipods is related to temperature (*e.g.* Steele and Steele, 1973; Borowsky, 1980; Fredette and Diaz, 1986; Takeuchi and Hirano, 1991). As for brood size, northern Gammaridean species usually produce smaller clutches during the colder months, in association with an increase in egg size, providing the offspring with a better chance of surviving harsh conditions (*e.g.* Van Dolah and Bird, 1980; Skadsheim, 1984; Pardal *et al*., 2000). The brood and embryo size in Gammarids is often reported as being proportional to the female’s body length (e.g. Beare and Moore, 1996; Costa and Costa, 1999; Cunha *et al*., 2000b) and limited by the amount of energy devoted by the female to reproduction, which depends on growth and maintenance costs (Sainte-Marie, 1991). Moreover, salinity–temperature

interactions have been shown to modify growth rates of a number of estuarine crustaceans (McKenney and Celestial, 1995).

Tolerances to low dissolved oxygen levels vary greatly among amphipods. Many are very sensitive to low levels, especially species restricted to areas where dissolved oxygen does not historically vary greatly. As for salinity, adult gammarids found in estuaries are fairly tolerant to a wide salinity range while many juveniles and embryos are not (Grosse *et al*., 1986).

1.2 Echinogammarus marinus

In the Mondego estuary, the observations of Marques *et al.* (1988) and Marques (1989) showed that only 18 of the 105 identified species of macroinvertebrates presented significant values of abundance and biomass. Four of those species were amphipods, including, *Echinogammarus marinus* (Leach, 1815). This gammarid presented an extensive distribution and abundance in the rocky substrates of the Mondego estuary and was, therefore, considered a key species in the algal covered habitat of *Fucus spp.*, where it can represent up to 40% of the macroinvertebrates biomass (Marques and Nogueira, 1991). Its known geographical distribution extends from Norway to Portugal, being, up until now, the Mondego estuary its southernmost limit (Maren, 1975*b*; Marques, 1989; Marques and Bellan-Santini, 1993).

This species is very well adapted to life in estuaries, consequence of being strongly eurihaline and able to withstand long emersion periods (Dorgelo, 1973; Pinkster and Broodbakker, 1980) and is usually found under *Fucus vesiculosus* (Phaeophyta, Fucales) in association with muddy deposits (Maren, 1975*b*). It is considered an important prey for the upper trophic levels, especially birds (Múrias *et al.*, 1996, 1997) and until recently it was thought that *E. marinus* individuals only fed on

green macroalgae. However, a study by Dick *et al.* (2005) suggested a more active predatory behaviour and indicated that *E. marinus* might capture and consume other macroinvertebrates (e.g. the isopod *Jaera nordmanni* and the oligochaete *Tubificoides benedii*), raising new issues to this amphipod's role in the community structure. Additionally, *E. marinus* individuals exhibited cannibalistic behavior in the laboratory, eating dead conspecifics and ingesting moulted exoskeleton, which supports the idea that this species requires a high nitrogen food source at times (Agnew and Moore, 1986).

1.3 Reference Studies

During the 80's, a study on the population dynamics of *Echinogammarus marinus* was carried out by Marques and Nogueira (1991) in the Mondego estuary. From October 1985 to September 1987, nine replicate samples were collected each month in three stations on the south arm of the estuary – Zostera, Pranto and Armazéns, respectively. This study showed clear seasonal variance in abundances, with peaks in spring and summer and also a spatial variation between sampling stations (1208.6, 1054.8 and 846.6 ind.m^{-2} for Zostera, Pranto and Armazéns, respectively). Sexual activity was found to be continuous throughout the year but recruitment was not, which allowed for the recognition of cohorts and subsequently the estimation of *E. marinus* lifespan (10 – 12 months) and growth production (6.36 to 8.81 g AFDW.m^{-2}.$year^{-1}$). Moreover, *E. marinus* presented a multivoltine life cycle with iteroparous females and high individual fecundity, revealing an **r** adaptive strategy expected in a warm temperate estuary as the Mondego estuary.

A new field study was carried out in the 90's by Maranhão *et al.* (2001) in the Mondego estuary from November 1994 to December 1995. Samples were taken

fortnightly in three stations, one in the north arm – Fontela – and two in the south arm – Pranto and Armazéns – with five replicates taken randomly at each sampling site. In this study abundance presented a very clear temporal variation with a winter peak only in the Pranto station and a spring peak in all stations. Abundance also presented spatial variation, with the Fontela station having the lowest values, whereas the upstream areas of the south arm (Pranto) reached the highest values. The population was sexually active throughout the year with continuous recruitment, which did not allow the recognition of cohorts, as opposed to the previous study by Marques and Nogueira (1991). Sex-ratio favoured females in the north arm during autumn and winter while males were dominant during spring and summer. As for the south arm males were almost always more abundant than females. Annual production was estimated using the Hynes average cohort method and showed the highest values for the Pranto station (3.85 – 5.38 g AFDW.m^{-2}.$year^{-1}$) and the lowest for the Fontela station (1.74 – 2.44 g AFDW.m^{-2}.$year^{-1}$). This study concluded that the population dynamics and production were affected by the hydraulic regime and that temperature, salinity and green macroalgae biomass were major factors influencing the *E. marinus* population.

Maranhão and Marques (2003) also published a laboratory experiment where they tested the influence of temperature and salinity on the duration of embryonic development, fecundity and growth of *Echinogammarus marinus*. In this study the organisms were exposed to different temperature-salinity combinations, respectively 10, 15 and 20ºC and 10, 15, 20 and 25‰. The results showed a decrease in the duration of embryonic development with a temperature rise from 10-15ºC to 20ºC (33-32 to 17 days respectively) whilst the highest production of juveniles was observed at 10ºC and the lowest at 20ºC. These results are opposite other studies reporting field data, where most northern gammaridean species produce smaller clutches during colder months to

provide the offspring with a better chance of survival (Van Dolah and Bird, 1980; Kolding and Fenchel, 1981; Skadsheim, 1984; Pardal *et al.*, 2000) but explained by the stable laboratory conditions that favoured the production of larger broods.

1.4 Temperature and Salinity

Estuaries are characterized by fluctuating conditions of salinity and temperature to the extent that both are considered dominant "ecological master factors", which may act either singly or in concert to modify the structure, function, and distribution of estuarine organisms (McKenney, 1996). Temperature, through its control of metabolic rates and linked effects on reproductive effort, and salinity, through its effect on molting and reproductive success, may be important physiological constraints to reproduction and determinants of life-history patterns of amphipods in estuaries (Cunha *et al.*, 2000a). Previous studies on the biology, population dynamics, and productivity of *E. marinus* (Marques and Nogueira, 1991; Maranhão *et al.*, 2001) showed that density, percentage of ovigerous females, sex ratio, fecundity, and egg volume were affected by temperature and salinity. The salinity and temperature ranges of this species in Mondego estuary were 4–31‰ and 7–29 °C, respectively (Marques and Nogueira, 1991; Maranhão *et al.*, 2001).

1.5 Intersexuality

Intersexuality is an abnormal condition where otherwise normal gonochoristic species possess both male and female characteristics, which has been reported throughout the animal kingdom (Reinboth, 1975). It is a very common feature in crustaceans, among which a large number of amphipod species inhabiting marine, estuarine and freshwater environments, covering artic, temperate and mediterranean

climates (e.g. Sars, 1895; Sexton and Huxley, 1921; Dunn *et al.*, 1990; Landewig *et al.*, 2003; Ford and Fernandes, 2005).

The causes of intersexuality are still partially unknown but it has been suggested to be the product of several factors, including microsporidian parasitism (Ford et al., 2004), environmental sex determination (ESD) (Dunn *et al.*, 1993) and pollution (Barbeau & Grecian, 2003; Ford *et al.*, 2004a). Ford and co-workers (2006) also suggested that pollution might enhance parasitism at anthropogenic impacted sites, while ESD is the probable cause of intersexuality at reference sites. In Amphipods, intersex phenotypes can be only female, male or both, depending on the species (Dunn *et al.*, 1990; Ladewig *et al.*, 2002; Barbeau and Grecian, 2003; Ford *et al.*, 2003). Ford and Fernandes (2005) observed that dual gender intersexuality occurred mostly in species that inhabit a range of salinities, which might indicate a variation in the tolerance and efficiency of parasites.

The incidence of intersexuality varies between species and within populations of the same species. Intersexuality poses several fitness costs, including smaller brood sizes, lower embryo survival, delayed maturation and reduced pairing success (Dunn *et al.*, 1993; Ford *et al.*, 2003, 2004b). Nevertheless, Miller and Buikema (1976) have described 100% intersexes of ovigerous females in populations of *Gammarus minus*. These costs differ according to sex-ratio fluctuations, which are common in amphipod populations, including *E. marinus* (Vlasblom, 1969). Populations with high incidence of intersexuality tend to be female-biased, probably linked to microsporidian feminizing parasites (Ford *et al.*, 2006). Moreover, a model used by Martins and co-workers (2009) suggests that strongly female biased populations are less sensitive to increases on intersexuality incidence, which could explain the persistance of populations with intersexed individuals, which are less fit that normal ones. However, if the reproduction

rate of intersex females is considerably lower than that of normal females, sensitivity to intersexuality will increase even if the sex ratio remains female-biased.

In the Mondego estuary, Pastorinho and co-workers (2009) referred the occurrence of intersexuality in *Echinogammarus marinus* individuals with an incidence rate of 14.3%. These findings presented new questions as to the consequences of such elevated percentage of intersexuality in the dynamics of *E. marinus* populations and a rather interesting path to explore in terms of modeling and potential extreme scenarios predictions (Guerra *et al.*, submitted).

1.6 Objectives

Given that amphipods are of extreme importance to estuarine trophic chains and that *Echinogammarus marinus* is the most abundant species in the hard substrata intertidal communities of the Mondego estuary, we aimed to:

1) Study the population dynamics and productivity of three different populations of *Echinogammarus marinus* and compare it with results from the 1980's and 1990's;

2) Detect the main biotic and abiotic factors that influence the *Echinogammarus marinus* population dynamics and productivity;

3) Evaluate the incidence of intersexuality in the studied populations and its impacts on the population structure, dynamics and productivity.

CHAPTER 2

MATERIAL AND METHODS

2. MATERIAL AND METHODS

2.1 Study Site

The present work took place in the Mondego estuary (40°08' N, 8°50' W), which is located on the Atlantic coast of Portugal in a warm temperate. The Mondego estuary consists of two arms, north and south that bifurcate at 7 km upstream from the estuary mouth. The two estuarine arms surround the Murraceira Island and reconnect again at about 1 km from the mouth of the estuary. This bifurcation causes the two arms to have distinct hydrographic characteristics. Thus, the north arm, where the Figueira da Foz harbor is located, is deeper (5 to 10m during high tide) while the south arm is almost silted up in the upstream areas (2 to 4m during high tide). This forces the freshwater to flow essentially through the north arm and makes it the main navigation channel, with consequent continuous dredging interventions to deepen the canal and normalize the margins, in order to improve navigation. Moreover, because the north arm is deeper, there is a faster penetration of the tide, causing daily changes in salinity to be much stronger, whilst the south arm presents higher daily temperature changes. The water circulation on the south arm is mainly dependent on the tides and a small freshwater input of a tributary, the Pranto river, which is artificially controlled by a sluice located 3km upstream to the confluence with the south arm (Marques, 1989; Marques *et al.*, 1993 a, b).

Both arms of the estuary have hard substrates covered primarily by *Fucus spp.*, constituting an eulitoral macroalgal belt. These hard substrates result from human activities and comprise up to 60% of the margins, representing an important portion of the intertidal area of the Mondego estuary (Marques and Nogueira, 1991). In the south arm, *Spartina maritima* and *Zostera noltii* marshes are among the richest areas with

regard to macrofauna abundance, biodiversity and productivity (Marques *et al*., 1993 b, Pardal, 1998, Dolbeth *et al*., 2003). *Zostera noltii* also constitutes, along with phytoplankton, microphytobenthos and green macroalgae, the main primary producers on the Mondego estuary (Cardoso *et al.*, 2004*a*). However, frequent blooms of *Ulva spp.* (formerly known as *Enteromorpha spp.*) have occurred in the south arm of this system for the past 20 years (Marques *et al.*, 1997; Flindt *et al.*, 1997a; Pardal, 1998; Lillebø *et al.*, 1999; Martins, 2000). These blooms were related not only to excessive nutrient discharge but also to the variation of salinity, light extinction coefficient and river hydrodynamics (Martins *et al.*, 2001). Depending on the frequency and extension of macroalgae blooms, the resulting eutrophication process might cause a shift on the dominant benthic primary producers, affecting the structure and functioning of the trophic chain and ultimately the species composition in the community (Marques *et al.*, 1993*b*).

i. The Mondego Estuary in the 80's

Since the time human activities took place on the Mondego estuary that significant changes have occurred on its structure and hydrological characteristics. Unfortunately, the data available goes only as far behind as the mid 80's so that will be the starting point of the present work.

In two studies carried out from 1986 to1990 by Marques *et al.* (1993*a*, *b*), the Mondego estuary was described as having a severe increase of environmental stress mainly due to harbor facilities and dredging activities that cause physical disturbance of the bottoms, industrial activities, salt works, aquaculture farms and nutrient discharges from agricultural fields in the upstream river valley. The physical disturbance occurred mostly on the north arm where biodiversity and structure of macrobenthic communities

was lower, whereas in the south arm the macrofaunal abundance and biodiversity was higher, especially in the *Zostera noltii* beds. The south arm was also less impacted by human activities, however frequent macroalgal blooms led to eutrophication problems and by the end of the 80's the area occupied by *Zostera noltii* was reduced from 15 ha to 1.6 ha (Cardoso *et al.*, 2005, 2008*a*; Verdelhos *et al.*, 2005).

ii. The Mondego Estuary in the 90's

During the 90's the anthropogenic pressures became even more accentuated. The communication between both arms was interrupted in order to regularize the main navigation channel (north arm) which almost ceased the supply of freshwater from the Mondego river to the south arm. Consequently, the water residence time was higher in the south arm, allowing for the deposition of sediments and increasing the light penetration in the water column as well as salinity. These conditions favored the occurrence of macroalgae blooms, especially of *Ulva spp.* (Marques *et al*., 1997; Flindt *et al*., 1997*a*; Pardal, 1998; Lillebø *et al*., 1999; Martins, 2000; Martins *et al.*, 2001; Duarte *et al.*, 2001) and the subsequent eutrophication process contributed to the reduction of the area covered by *Zostera noltii* to 0.02 ha (Marques *et al.*, 2003; Cardoso *et al.*, 2004; Neto, 2004).

In 1998, several mitigation measures were implemented and gradually allowed for the system's recovery from the effects of eutrophication. These included (1) the partial re-establishment of the connection between both arms, improving freshwater circulation and reducing the nutrient load; (2) rerouting of most of the Pranto nutrient enriched freshwater to the north arm, leading to nutrient loading reduction; (3) protection of the seagrass beds from human disturbance and (4) public awareness to the ecological importance of intertidal vegetation for the system's health and related socio-

economic activities of the estuary (Cardoso *et al.*, 2005; Lillebø *et al.*, 2005; Verdelhos *et al.*, 2005).

iii. The Mondego Estuary nowadays

Presently, the Mondego estuary continues to suffer important anthropogenic impacts related to tourism, harbor activities, aquaculture and agriculture from the upstream rice farms (Pinto *et al.*, 2010).

The mitigation measures that took place in the late 90's seem to have been partially effective, since the nutrient loading was reduced (Cardoso *et al.*, 2010) and no extensive macroalgae blooms were recorded ever since. This allowed *Zostera noltii* to recover, at least in spatial extent (4.7 ha in 2006), although biomass is still much lower than in 1993 but with positive impacts on the macrobenthic community structure. (Dolbeth *et al.*, 2011).

Further interventions took place in 2006, when the complete re-establishment of the communication between both arms of the Mondego estuary occurred (Marques *et al.*, 2007; Veríssimo *et al.*, in press). However, from 2007 to 2008, the works of Gala Bridge expansion took place, and although it was a small-scale intervention downstream, it probably had a negative impact on the system and masked the effectiveness of the management intervention at the subtidal macrobenthic community level (Veríssimo *et al.*, in press).

Extreme climate events have also occurred during the last decade, a major flood took place in the winter of 2000/2001 and heat waves were recorded in 2003 and 2005, the latter accompanied by a severe drought (Neto *et al.*, 2010; Dolbeth *et al.*, 2011), which had negative impacts specially on the south arm macrobenthic communities. (Dolbeth *et al.*, 2011).

2.2. Sampling Strategy

In order to have feasible comparisons with the previous studies, we followed a similar sampling strategy described by Marques and Nogueira (1991) and Maranhão *et al.* (2001). *Echinogammarus marinus* populations were sampled during 12 months, from April 2009 to March 2010, at three different sampling stations, one located in the north arm and the other two located in the south arm of the estuary, representing different environmental conditions for *E. marinus*. The samples were collected once a month, during the low tide, to facilitate the access to the sampling areas.

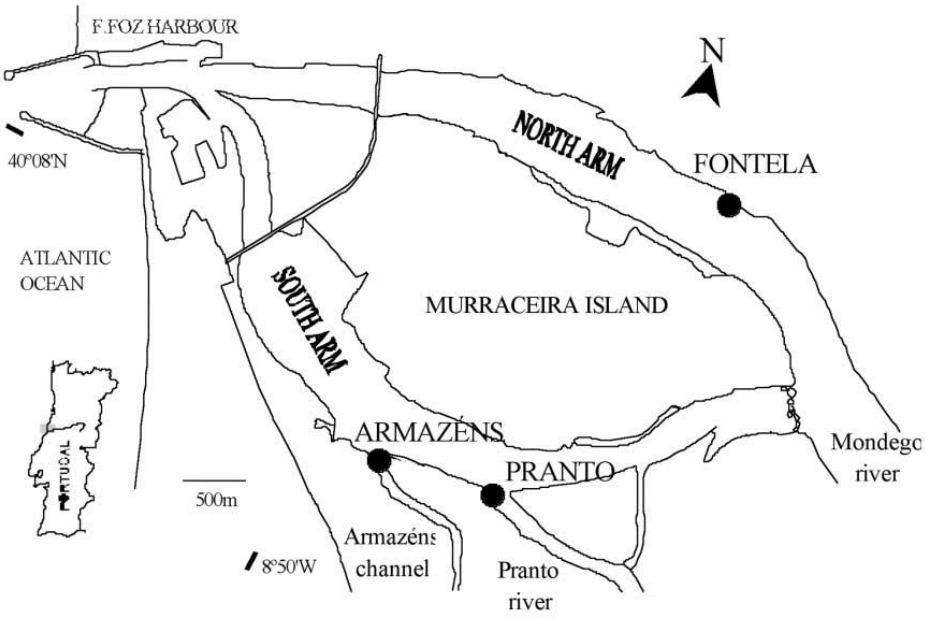


Figure 1 – Mondego estuary with the localization of the sampling stations. (Maranhão *et al.*, 2001)

The sampling station in the north arm – FONTELA – is located upstream from Figueira da Foz commercial harbour and is characterised by coarse sandy to muddy sediments with disperse stones and an average content of organic matter of 1.92% (Maranhão, 2002).

The two stations in the south arm – ARMAZÉNS and PRANTO – are morphologically similar and characterised by a wide area covered in *Fucus vesiculosus* on the rocky margins with high abundances of *E.marinus* (Marques, 1989). The Armazéns station is located in the left margin of this arm and is mainly characterized by some disperse stones and muddy sediment, with an average organic matter content of 5.47% (Maranhão, 2002)**.** The Pranto station is located upstream from the Armazéns station, at the mouth of the Pranto river, also on the left margin of the south arm and is mostly constituted by muddy sediments presenting an average organic matter content of 3.25% (Duarte, 1990; Maranhão, 2002).

2.2.1 Field Procedure

Three replicates were randomly taken at each sampling site. Since the sampled surface was often irregular, the area was estimated by projecting its shape to a polyethylene sheet, which was later weighed for weight-area conversion. Since several types of polyethylene sheets were used, a weight-area ratio was determined for each type, in order to exclude possible errors due to differences in the weight of different sheets.

Each replicate was taken by removing all the algae present in each area, since most adult amphipods take shelter between the fronds of the algae, scraping the rocky surface where the algae were attached and removing a superficial 2 cm layer around the rocks to ensure that all amphipods were collected. Both the scraped and the superficial 2 cm layer of sediment were washed *in situ* using a 0.5 mm nylon mesh, ideal to retain even the smaller *E. marinus* individuals (Marques and Nogueira, 1991), while the remaining algae were washed afterwards in the lab.

In every sampling station temperature, salinity, dissolved oxygen and pH were measured *in situ*. A water sample for each station was collected from low water retention pools near the sampled algae, to determine the dissolved nutrient content of nitrites, nitrates, phosphates and ammonia. Sediment samples were also taken in order to determine the organic matter content of the sediment.

2.2.2 Laboratory Procedure

The part of the samples previously washed in the field was preserved in 4% formalin. The other part of the samples, containing most of the *Fucus spp.*, was carefully washed with tap water to remove all *E. marinus* individuals. Subsequently, *Fucus spp.* was weighted to determine its fresh weight, dried in a stove at 60ºC for at least 72h in order to determine its dry weight for further biomass estimation. Some samples of *Fucus* were also burned in a muffle at 450ºC for 8h to ensure determination of the ash-free dry weight (AFDW). All amphipods were preserved in 80% ethanol until further identification. A similar procedure was followed with the sediment samples, to estimate the organic matter content of the sediment.

Water samples were filtered upon arrival on the laboratory (Whatman GF/F glass-fibre filter) and stored frozen at -18ºC until analysis. Nutrient analysis was performed according to the standard methods described in Limnologisk Metodik (1992) for ammonia (N-NH3) and phosphate (P-PO4), and in Strickland and Parsons (1972) for nitrate (N-NO3) and nitrite (N-NO2).

After sorting, the amphipods were identified but only the *Echinogammarus marinus* species was considered. Individuals were counted and the cephalic length was measured to the nearest 0.02 mm, between the extremity of the rostrum and the base of the head, using a calibrated stereomicroscope. Measuring the cephalic length is more

convenient than the total length because the comma-shaped bodies of *E. marinus* make it harder to determine age and growth (Marques and Nogueira, 1991).

The sex of each individual was determined based on the presence or absence of female characters, oostegites and/or broods and male characters, genital papillae. Females were also classified as mature and immature, thus considering an immature female as having a cephalic length (C_L) lower than the smallest brooding female (C_L < 1,02 mm). Individuals without these features were considered to be juveniles and individuals with both features were classified as intersex males, if they had genital papillae and rudimentary brood plates, or intersex females if the less developed character were the genital papillae (Ford *et al.* 2003).

In the presence of brooding females, the eggs were removed and counted to estimate fecundity and examined in order to determine the development stage. Five stages were considered, based on the criteria described by Skadsheim (1982) and adapted by Marques and Nogueira (1991), respectively:

- **A**, newly laid eggs, spherical grouped and resembling a gelatinous mass;
- **B**, eggs well separated, oblong internally homogeneous;
- **C**, embryo comma-shape with vestigial pereopods already visible;
- **D**, constriction of the comma clearly visible, appendages segmented and looking slender, eyes visible, cephalotorax orange-red;
- **E**, hatched and free juveniles (these may stay some days in the brood pouch).

Due to some difficulties in distinguishing stages A and B, data regarding these two stages were pooled in an AB stage.

2.2.3 Data Analysis

2.2.3.1 Density, biomass and production estimation

Total length (T_L) and weight (W) of the *Echinogammarus marinus* individuals were obtained through allometric equations, previously determined by Marques and Nogueira, 1991, respectively:

$$T_L = -1.211995 + 10.668509\, C_L$$

$$W = 1.592924 \times C_L^{3.94344}$$

where **W** refers to ash-free dry weight (mg AFDW) and $\mathbf{C_L}$ is the cephalic length (mm).

The abundance/density (D) of *E. marinus* in the algae cover (number of individuals.m^{-2}) was related with both sampled area and algal density through the formula D = A*B, where A is the number of amphipods per gram of algae in each sample and B is the mean grams of algae per square meter on a sample date (Marques and Nogueira, 1991).

Secondary production was calculated with the sum of size frequency technique (posterior to Krueger and Martin, 1980), modified to estimate the production in intervals between samples. This appears to be the most suitable technique since, similar to the study by Maranhão *et al.* (2001), the present study showed that *E. marinus* populations in the Mondego estuary have continuous recruitment, making it impossible to identify cohorts.

Firstly, this method comprises the evaluation of the total number of individuals that developed in each size class. Production is then considered as the sum of the biomass increments of different size classes for a given period. Therefore, production is given by:

$$P = \sum_{j=1}^{a-1} 0.5\,[(Y_{i,j} + Y_{i+1,j}) - (Y_{i,j+1} + Y_{i+1,j+1})] \,.\, (W_j \,.\, W_{j+1})^{0.5} .\, (t)^{-1}$$

where **P** is the production rate, $\mathbf{Y_{i,j}}$ is the mean density (ind.m^{-2}) of the size class j on the date i, $\mathbf{W_j}$ is the biomass (g.m^{-2}) of the size class j and **t** stands for the time (in days) between the dates i and i+1.

Fucus spp. biomass was determined by its dry weight, which in turn was estimated through fresh weight (W_F) – dry weight (W_D) regressions mentioned in the Laboratory Procedure paragraph. Dry weight corresponded, in mean, to approximately 14% (± standard deviation) of the fresh weight (N=25), as shown by the equation:

$$W_D = 0.13875\ (\pm 0.06)\,.\,W_F$$

2.2.3.2 Univariate Analysis

Density and biomass data were subjected to individual univariate analyses with PRIMER-E's software PRIMER 6 + PERMANOVA© software (software package from Plymouth Marine Laboratory, UK) (Clarke, 2001; Anderson *et al.*, 2008). The data was transformed by square root and then analysed by a principal coordinate analysis (PCO) through the Bray-Curtis similarity measure. A factorial PERMANOVA was also applied (significance level of $\alpha = 0.05$; 9999 permutations with Monte Carlo test), with two fixed factors, "station" (comprising three levels: pranto, armazéns and fontela) and "season" (including four levels: spring, summer, autumn and winter), to check for significant differences between stations, seasons and a possible interaction. Pair-wise tests (significance level of $\alpha = 0.05$; 9999 permutations with Monte Carlo test) were performed to test for differences within the levels of each factor.

2.2.3.3 Multivariate Analysis

A principal component analysis (PCA) was performed on the physicochemical data, after square root transformation (nutrient data only) and normalization, in order to determine which variables contributed for the variation between sampling stations and seasons. The relationships between the environmental variables and the density variation were explored through a distance based linear model (DistLM), with “Best” as the selection procedure and “BIC” as the selection criterion. A distance based redundancy analysis (dbRDA) was performed to have a representation of the model in the multivariate space.

CHAPTER 3

RESULTS

3. RESULTS

3.1 Environmental factors

Throughout the study period, temporal and spatial differences in the physicochemical conditions were observed in the Mondego estuary. Water temperature and salinity (Fig. 2A and 2B, respectively) seemed to have been specially influenced by seasonality, with maximum values during summer and lowest in the winter. Spatial differences in the water temperature are probably related to the sampling strategy, since the south arm stations (Pranto and Armazéns) were sampled first and the north arm station (Fontela) was always the last one to be sampled, 2h after the other two. Dissolved oxygen (Fig. 2C) also presented seasonal variation during the study period, with highest values during spring. Fontela always showed higher values although, as mentioned before, some bias related to the sampling strategy must be taken into account. The pH values (Fig. 2D) oscillated mainly in the spring and summer months. However, doubtful values due to the probe malfunctioning in the field prevent a full consideration of the data and raises concerns as to its validity.

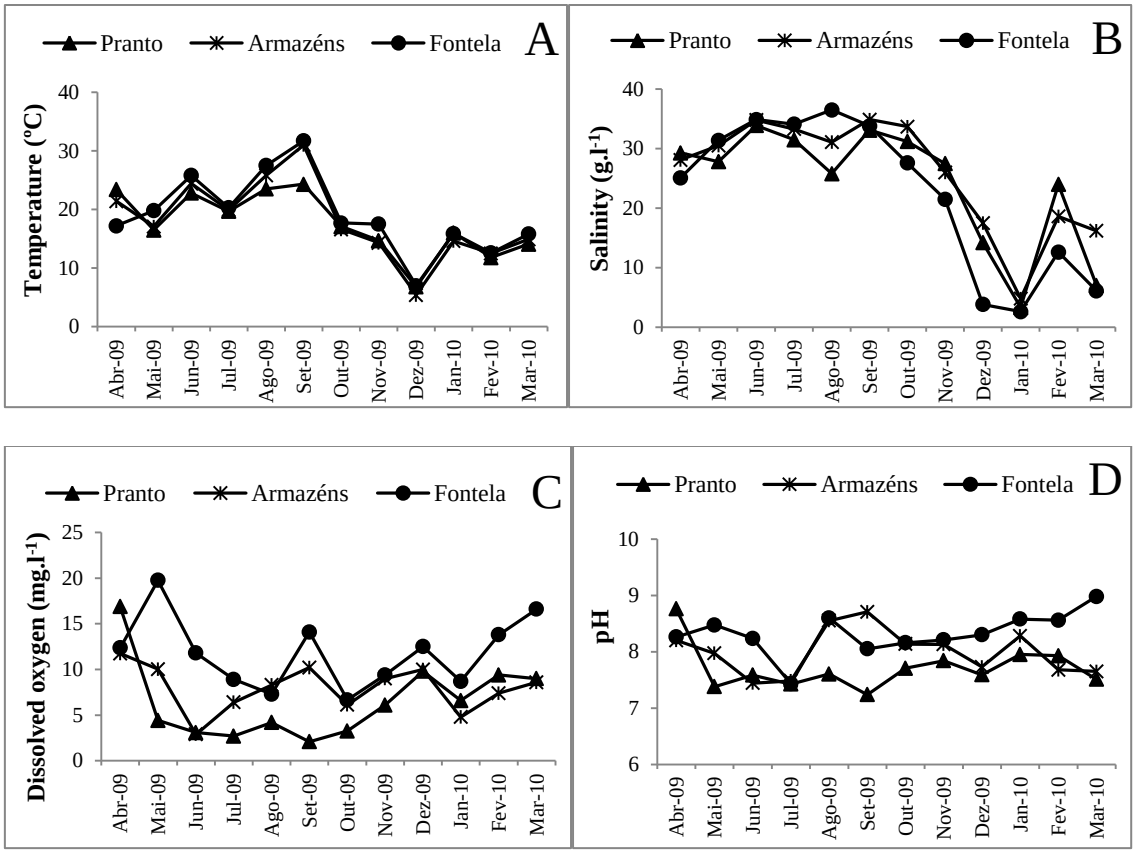
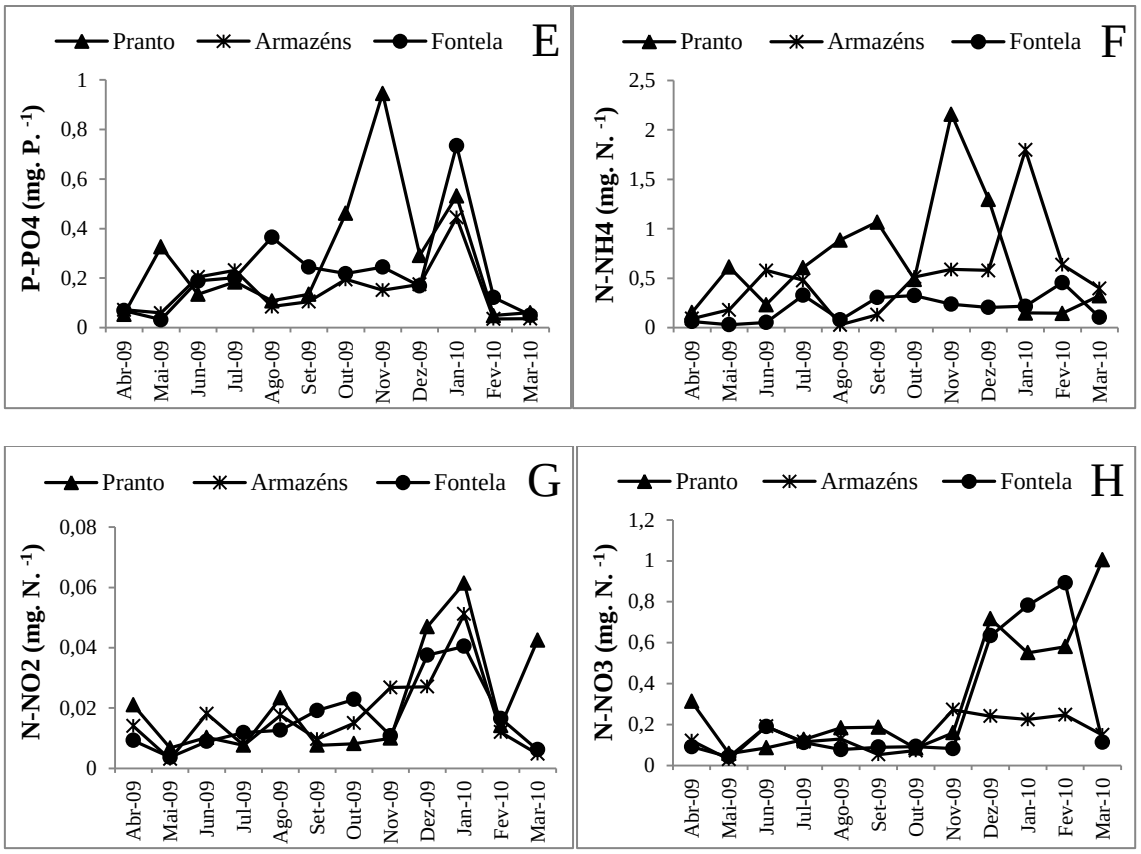


Figure 2 – Variation of temperature (A), salinity (B), dissolved oxygen (C) and pH (D) during the study period, at the three sampling sites.

Nutrient analysis results showed considerable ammonia oscillations in the south arm, with autumn and winter peaks in Pranto and Armazéns, respectively (Fig. 3F). Phosphate concentrations were relatively stable throughout the year apart from Pranto station and a small winter peak in all sampling sites. Pranto presented great fluctuations, with a high summer peak, common to temperate estuaries such as the Mondego (Pardal, 1998) and a smaller autumn peak (Fig. 3E). Nitrite and nitrate concentrations (Fig 3G and 3H, respectively) showed seasonal variation in all sampling stations, reaching the highest values during autumn and winter. This time of the year coincides with an increase in precipitation which generally leads to the wash-out of nutrient enriched water from the upstream agricultural fields. As for the organic matter content of the

sediment (Fig. 3I), Armazéns presented the highest content, with an average of 3.2%, while the coarse sediments of Fontela registered the lowest value, with an average of 1.7% of organic matter content. At Pranto's sampling station, organic matter content averaged 2.8%, although it reached higher values than Armazéns in some months and lower values than Fontela in others.



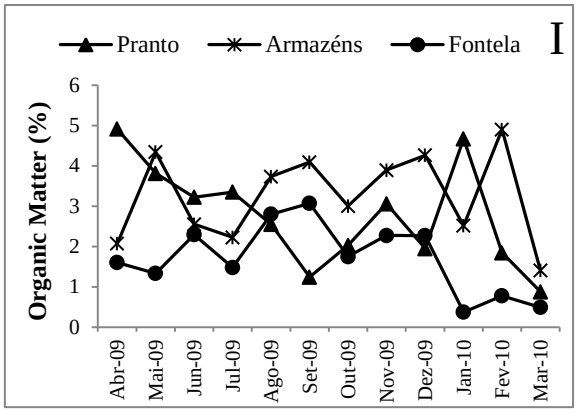


Figure 3 – Variation dissolved inorganic nutrients, P-PO4 (E), N-NH4 (F), N-NO2 (G), N-NO3 (H) and organic matter content in the sediment (I), during the study period, at the three sampling sites.

Principal component analysis (PCA) showed a pattern between different seasons (Fig. 4A), with a clear separation between spring and summer samples and the winter samples, while autumn samples are more disperse on the plot. A separation between sampling stations was also found (Fig. 4B), although not as clear as for seasonality, with Armazéns and Fontela spreading mainly along the PC1, while Pranto distribution was predominantly alongside the PC2. For both PCAs, the first two axes accounted for 55.5% of the total variation, with the fist axis explaining 30.5% and the second axis 25%. On the first axis, temperature and salinity had a positive influence whereas nitrite and nitrate had a negative influence. On the second axis, ammonia and phosphate showed a strong positive contribution while dissolved oxygen and pH had a negative influence. Apparently, pH and organic matter content were not influenced by season or sampling station. Considering seasonality, temperature and salinity seem to be the most prevalent factors in spring and summer, while nitrite and nitrate appear to have a greater influence in winter.

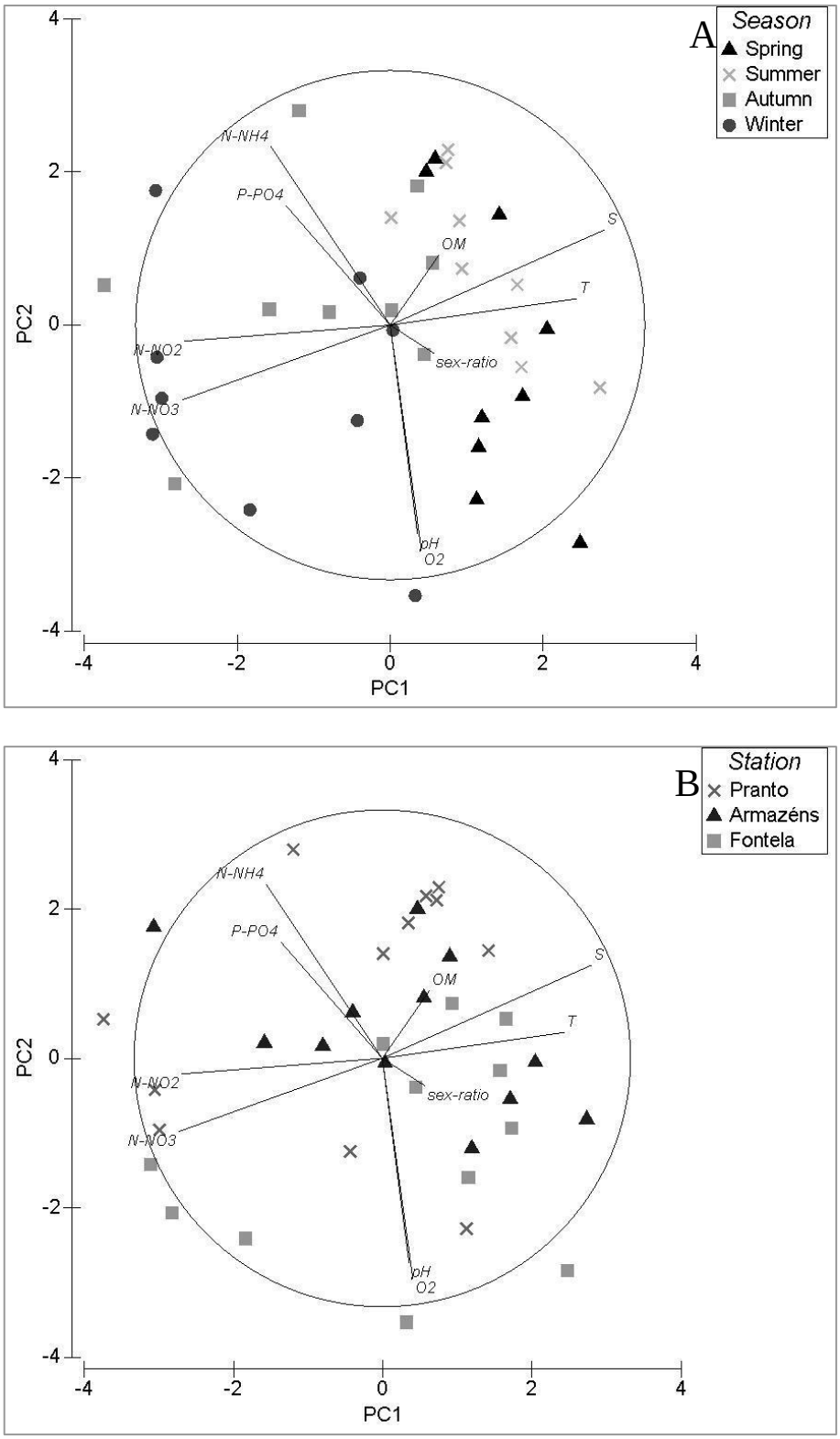


Figure 4 – Two dimensional PCA of the abiotic variables in relation to Station (A) and Season (B). (Axis 1 = 30.5% variation; Axis 2 = 20% variation). S – salinity; T – temperature; O_2 – dissolved oxygen ($mg.l^{-1}$); OM – organic matter content; N-NH4 – ammonia, N-NO2 – nitrite; N-NO3 – nitrate, P-PO4 – phosphate.

3.2 Density and Biomass

Density of *Echinogammarus marinus* (Fig. 5) changed seasonally with a spring peak in all stations but gradually decreasing throughout the study period. A smaller autumn peak also occurred but only in Fontela and Armazéns. The spatial distribution of *E. marinus* oscillated between sampling stations and the highest values were observed in Fontela whilst the lowest were registered in Pranto.

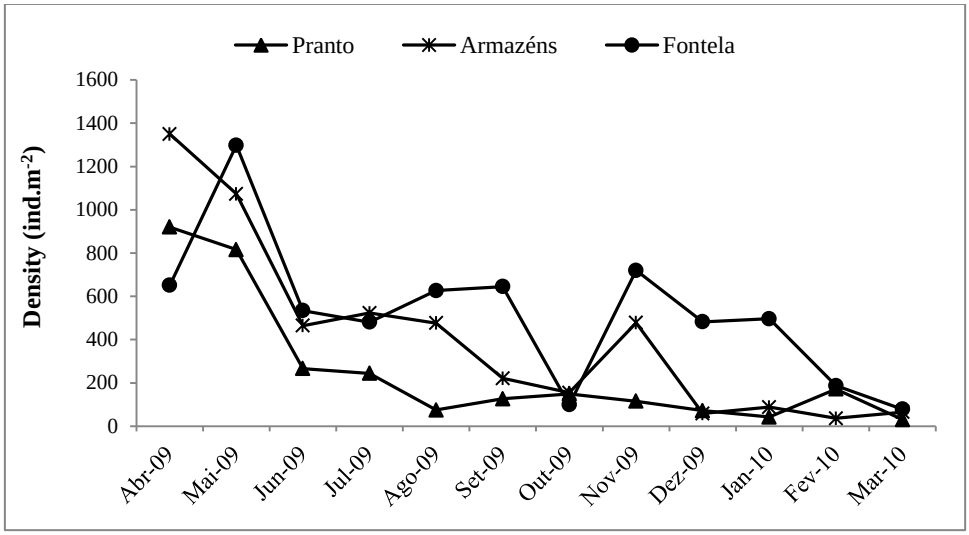


Figure 5 – Density of *Echinogammarus marinus* at the three sampling stations throughout the study period.

Biomass of *E. marinus* (Fig. 6) also changed seasonally, with higher values during spring in all stations but declined during the rest of the study period. The highest and lowest value was recorded for the Armazéns station, although Pranto showed lower values throughout the year.

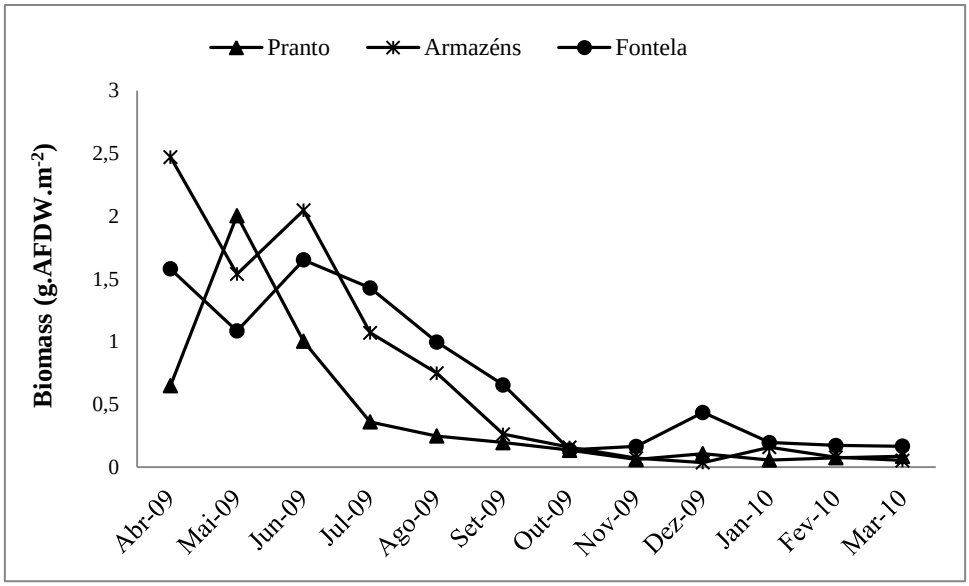


Figure 6 – Biomass of *Echinogammarus marinus* at the three sampling stations throughout the study period.

Density and biomass were also different between groups of *E. marinus*. Juveniles were always more abundant (Fig. 7A) in all sampling stations, followed by males and females with similar densities although slightly higher in males. Biomass (Fig. 7B) was generally higher in males during the study period and in all sampling stations, followed by females and juveniles. Intersexual individuals presented the lowest values for both density and biomass.

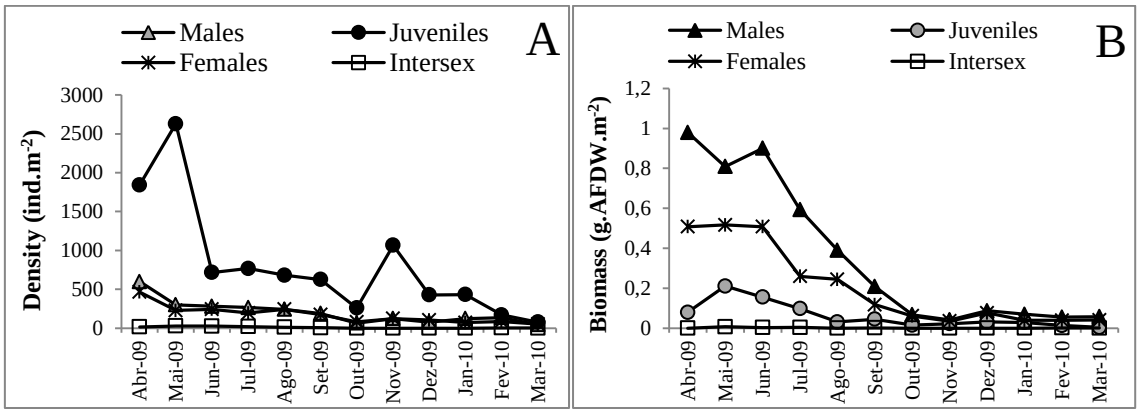


Figure 7 – Variation of density (A) and biomass (B) of juveniles, females, males and intersex individuals of E. marinus throughout the year, at three sampling stations of the Mondego estuary.

Regarding intersexuality (Table 1), results show a very low percentage of intersexuality incidence (0.1% total), considering the total of individuals sampled during the study period, and occurred in similar proportions in all sampling stations.

Table 1 – Intersexuality incidence in three different populations of the Mondego estuary, during the study period.

	Nº Individuals	**Intersexual Individuals**	**Intersexuality (%)**
Fontela	5539	5	0,09
Armazéns	5180	7	0,14
Pranto	3298	3	0,09
Total	14017	15	0,1

PERMANOVA showed temporal and spatial differences for both density and biomass of *Echinogammarus marinus*. Density analysis showed significant differences between stations ($F(St)_{2,24}=5.5758$; $p=0.0021$) and pair-wise tests demonstrated that Fontela is different than the other stations ($P(perm)<0.05$), although Pranto and Armazéns were not statistically different from each other ($P(perm)>0.05$). Differences between seasons were also detected ($F(St)_{3,24}=12.566$; $p=0.0001$) with the pair-wise tests indicating that all seasons are different from each other, except from autumn and winter ($P(perm)>0.05$). Regarding biomass, differences between stations were also observed ($F(St)_{2,24}=5.8871$; $p=0.0003$) with differences between Fontela and the other stations ($P(perm)<0.05$) but not between Pranto and Armazéns ($P(perm)>0.05$), as shown by pair-wise tests. Seasonality also influenced biomass ($F(St)_{3,24}=33.553$;

p=0.0001) and the pair-wise tests showed differences among all seasons (P(perm)<0.05), apart from autumn and winter (P(perm)>0.05).

Principal Coordinate Analysis (PCO) was performed for density and biomass data. Density PCO (Fig. 8) explained 91,8% of the total variation in two axis and apparently seasonality had a stronger effect over density than spatial distribution, with spring and summer having a positive contribution, whereas autumn and winter had a negative influence on density. Likewise, the biomass PCO (Fig. 9) suggests a stronger influence of the season factor rather than the station, showing the same pattern of negative influence of autumn/winter and positive contribution of spring/summer as observed for de density PCO.

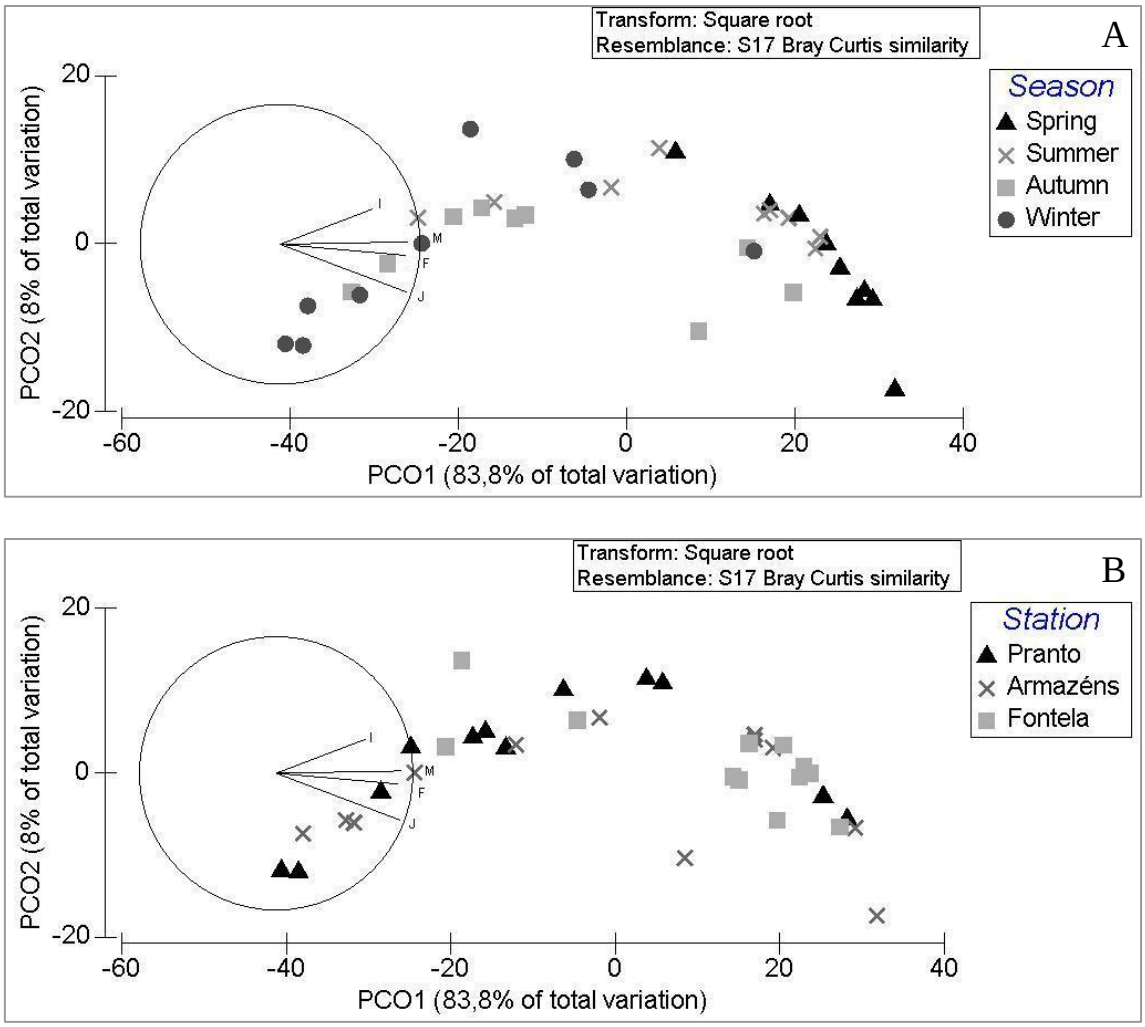


Figure 8 – PCO based on group density according to seasonality (A) and sampling station (B). I – intersex; M – males; F – females; J – juveniles.

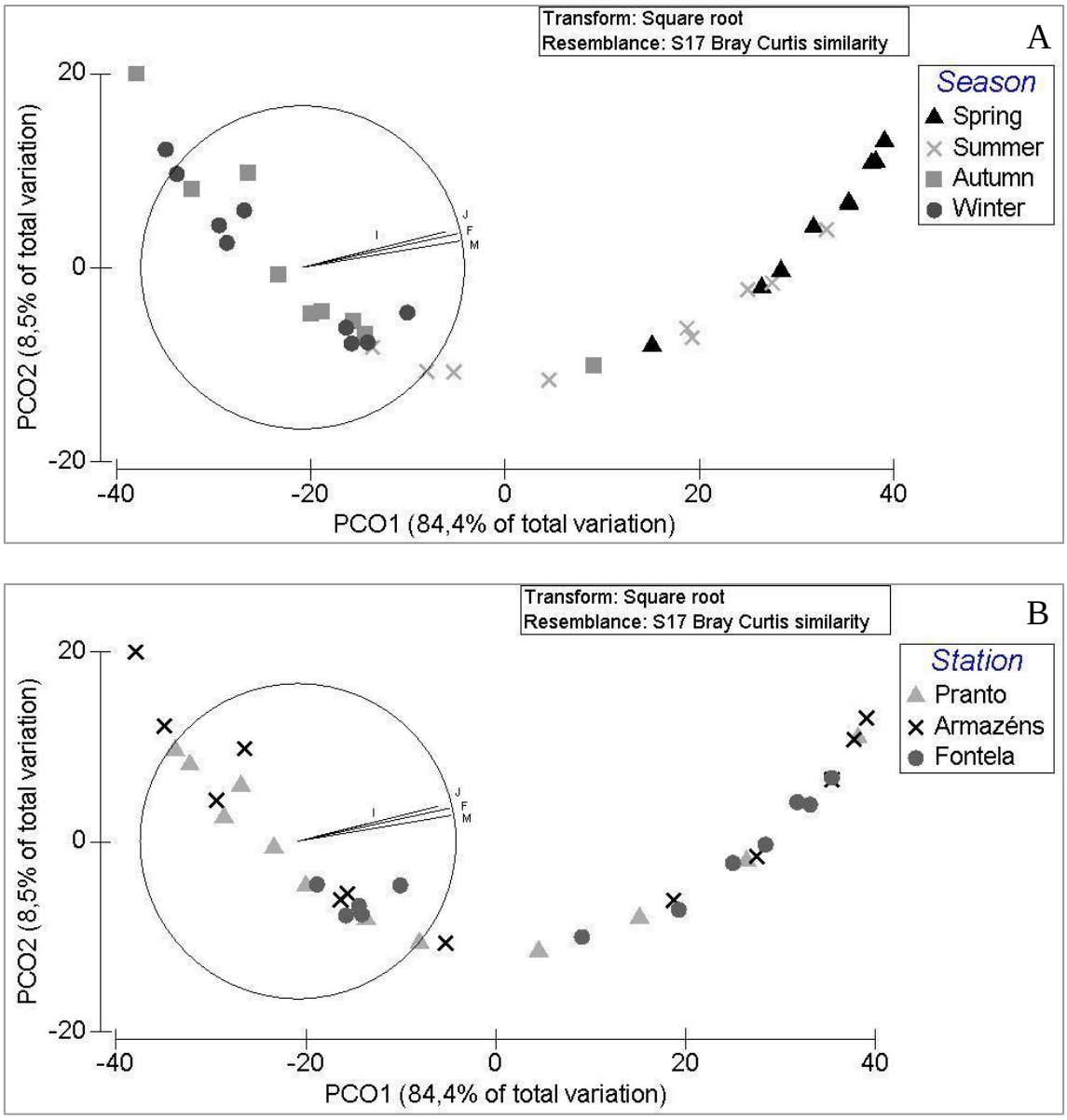


Figure 9 – PCO based on biomass in each season (A) and at each sampling station (B). I – intersex; J – juveniles; F – females; M – males.

3.3 Influence of environmental variables on *E. marinus* distribution

The DistLM analysis showed a best solution including one variable (R^2=0.24) that was salinity, and a second one (R^2=0.39) that included two variables, salinity and ammonia. The second model was chosen due to its higher R^2, although it only explains 39% of the variability. To visualize the regression model in a constrained ordination, a

distance based redundancy analysis (dbRDA) was performed (Fig. 10), resulting in a two dimensional plot where the first axis explains 96.4% variation percentage out of the fitted model and 51.4% variation percentage explained out of the total variation.

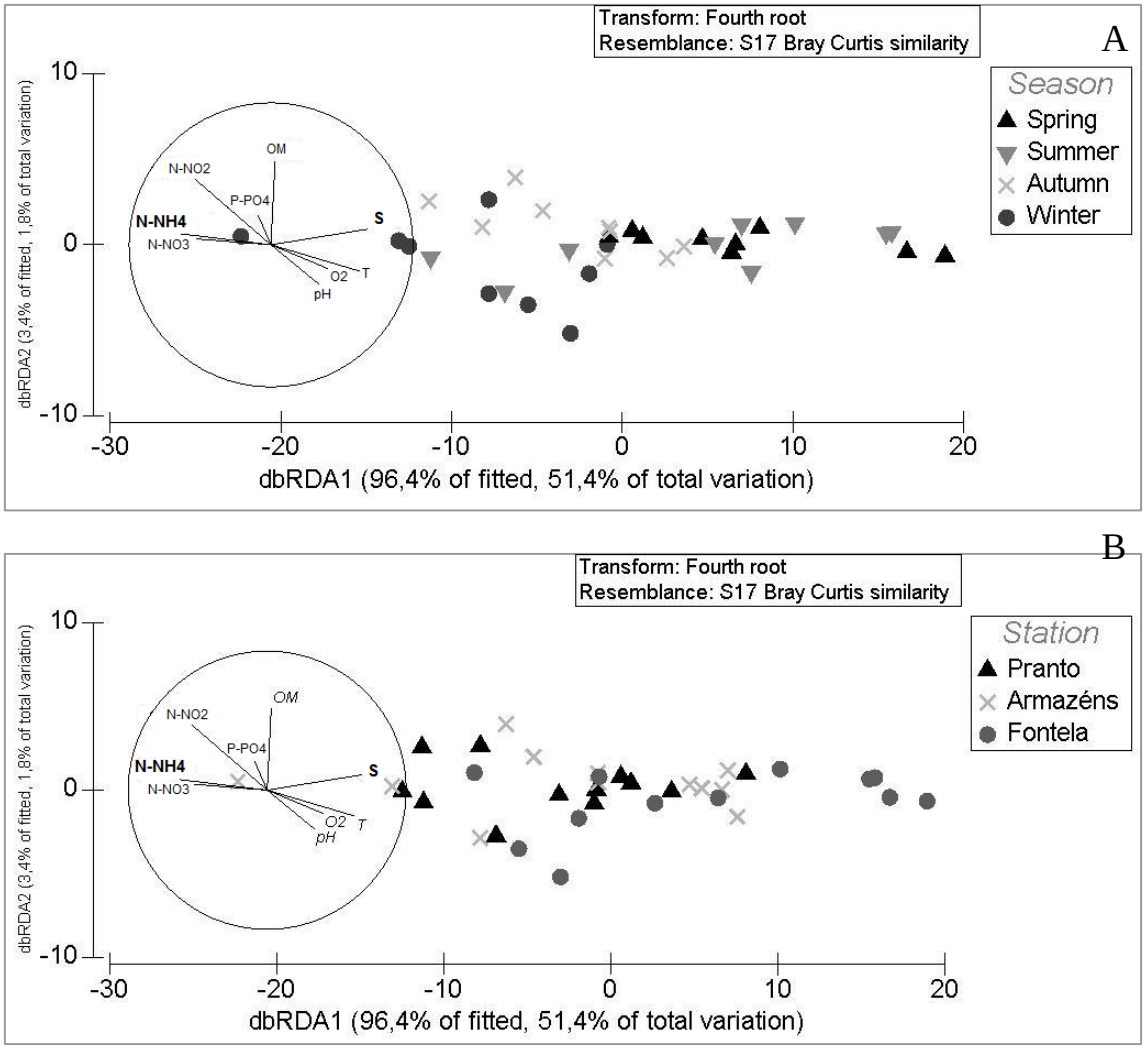


Figure 10 – dbRDA model for the interaction between environmental variables and *E. marinus* density, according to seasonality (A) and sampling station (B). N-NH4 – ammonia; S – salinity.

3.4 Reproduction and sex-ratio

In this study, the sexual activity of *E. marinus* populations was estimated through the percentage of ovigerous females in relation to the total number of females and the proportion of juveniles in the total population (Fig. 11). Our data indicates that

sexual activity was continuous during the study period in all sampling stations, although it almost ceased during winter in Fontela (Fig. 11F) and at the end of the winter in Pranto (Fig. 11P). The highest percentage of ovigerous females was observed in Fontela (71%), although, in average Armazéns and Pranto presented the higher percentages of ovigerous females (Fig. 11A and 11P, respectively). As expected, the variation of juveniles during the study period has followed the distribution of ovigerous females.

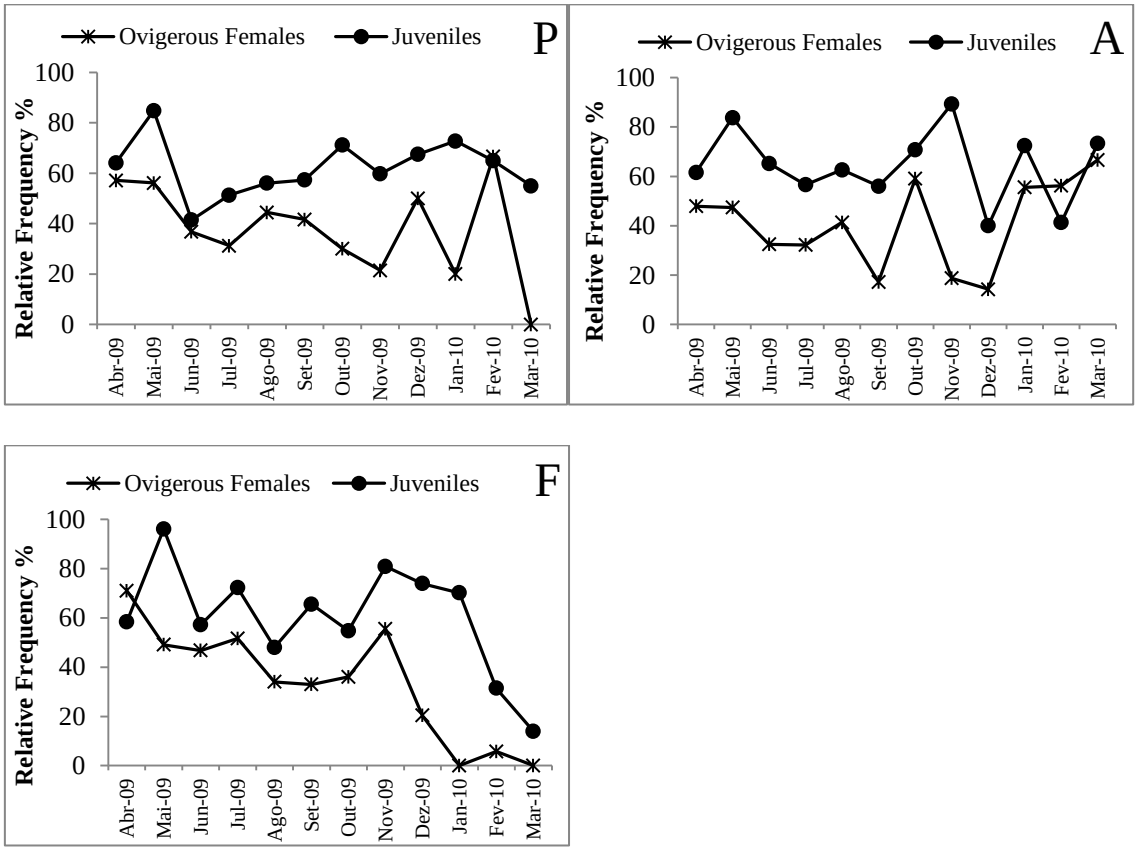


Figure 11 – Relative frequencies of Juveniles and Ovigerous Females in all sampling stations, throughout the study period. P – Pranto; A – Armazéns; F – Fontela.

Sex-ratio was not stable throughout the study period but was predominantly male in all stations (Fig. 12). Pranto and Armazéns (Fig. 12P, 12A and 12SA) seem to follow a similar sex-ratio pattern, shifting to female dominance during autumn, recovering during early winter and shifting again in late winter. In Fontela (Fig. 12F), females were dominant during the summer and early autumn. The highest proportion of males was observed in the winter in all stations.

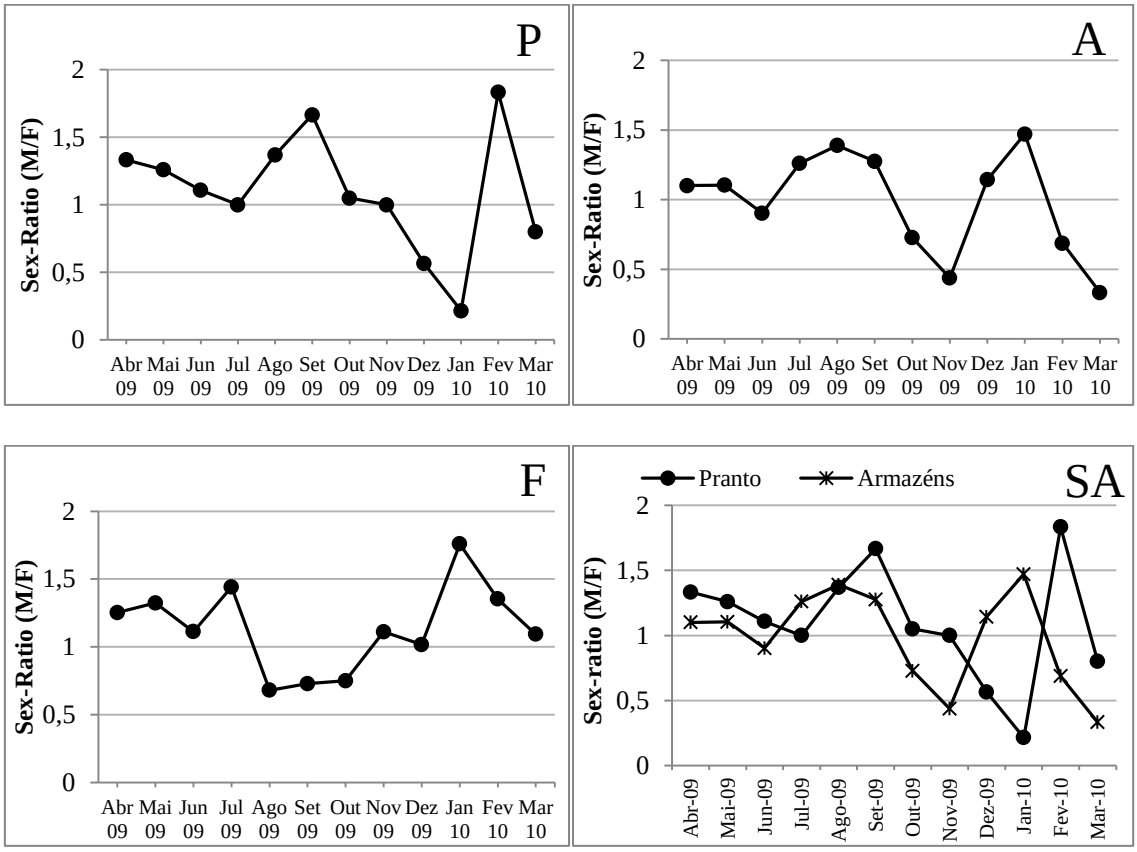


Figure 12 – Sex-ratio occurrence in all sampling stations, during the study period. P – Pranto, A – Armazéns; F – Fontela; SA – South Arm.

3.5 Production

Annual production (Table 2) was higher in Armazéns while the average annual biomass and annual P/$\overline{B}$ ratios were very similar in Fontela and Armazéns. Pranto presented the lowest values for annual production, average annual biomass and annual P/$\overline{B}$ ratio.

Table 2 – *Echinogammarus marinus* production estimates for the three sampling stations.

	P (g AFDW.m^{-2}.y^{-1})	**$\overline{B}$ (g.m^{-2})**	**P/$\overline{B}$**
Pranto	2.11	0.41	5.15
Armazéns	6.35	0.72	8.82
Fontela	5.78	0.72	8.03

CHAPTER 4

DISCUSSION

4. DISCUSSION

4.1 Density and biomass

Echinogammarus marinus density showed clear spatial and seasonal variations. The Fontela population registered the highest values of density and Pranto the lowest, although no significant differences were found between the sampling stations. Still, these results differ from the ones obtained by Maranhão and co-workers (2001) who observed higher densities in Pranto and lower in Fontela. Marques and Nogueira (1991) also found high densities in Pranto, even though the highest values were recorded in Armazéns. DistLM analysis indicated salinity and ammonia as the best environmental variables to explain the density variability, with a stronger positive influence of salinity over the observed variation, while ammonia showed a negative influence In fact ammonia concentrations were generally higher in Pranto than in Fontela, which might explain the lower densities found in Pranto. On the other hand, salinity showed a similar pattern for all sampling stations, although it was previously considered a key factor affecting the population dynamics of *Echinogammarus marinus*, through its effects on moulting and reproductive success (Marques and Nogueira, 1991; Maranhão *et al.*, 2001; Maranhão and Marques, 2003). Marques and Nogueira (1991) found a clear positive correlation between density and temperature and dissolved oxygen values. In this study, dissolved oxygen was higher in Fontela and lower in Pranto, still no correlation was found between this parameter and density and all assumptions must be regarded as speculative. Considering the study by Maranhão *et al.* (2001), a positive correlation was found between density and green macrolagae biomass in the Fontela and Armazéns stations, which is probably related as its use as a food source. Since green macroalgae biomass was not evaluated in the present work and it is considered as an

important food source for E. marinus, it must be assumed that a possible explanatory variable for the density variation was left out.

Seasonal variation was also clear, with the highest densities recorded during spring followed by a significant decrease, especially in Pranto, and a slight autumn recovery in Armazéns and Fontela. By the end of the study period, *E. marinus* densities had reached very low values in all sampling stations, although a possible population recovery was to be expected in the upcoming spring, as Marques and Nogueira (1991) observed in their two year study, but logistical constraints prevented a more prolonged study. Despite the Pranto station values, the seasonal variation is generally consistent with the previous studies on the Mondego estuary (Marques and Nogueira, 1991; Maranhão *et al.*, 2001), which also reported higher densities during spring and summer months.

The density ranges observed in this study (100-1400 ind.m^{-2}) increased in relation to the previous study by Maranhão and co-workers (2001) but are still far from the ranges described by Marques and Nogueira (1991), indicating that the system is slowly recovering from the multiple stressors that have occurred for the past twenty years in the Mondego estuary.

Regarding biomass, all sampling stations presented high values during spring and early summer, followed by a steep decrease until the end of the study period. This is in agreement with PCO analysis, which indicated that biomass increase is favoured in spring and summer contrarily to autumn and winter.

Considering population structure, the *E. marinus* populations were generally dominated by juveniles although during winter they presented similar densities to that of males and females, probably as the result of exposure to more harsh conditions. Both Marques and Nogueira (1991) and Maranhão *et al.*, (2001) reported a dominance of

juveniles over 50%, which was, apart from a few exceptions, the general pattern observed in this study. Biomass was considerably higher in males until the end of the summer, followed by females and juveniles. However, from the beginning of autumn and until late winter, all groups presented lower biomasses, probably related to a higher energy cost when exposed to more stressful conditions.

Intersexuality was one of the most stimulating assumptions in the beginning of this work, considering all the hypothetical consequences to the *Echinogammarus marinus* populations of the Mondego estuary of such an elevated percentage (14.3%) described by Pastorinho and co-workers (2009). Still, results have demonstrated a very low percentage of intersexual individuals (0.1%), considered insignificant to the population dynamics, given the total of individuals sampled (table I), which is in accordance with previous studies of *E. marinus* in the Mondego estuary (Marques and Nogueira, 1991; Maranhão *et al.*, 2001) and found no evidence of intersexual individuals.

4.2 Reproduction and Sex-Ratio

In the present study, the available data demonstrates that sexual activity was continuous throughout the year (Fig. 11) as well as the recruitment for the same period. Reproduction almost ceased during winter in Fontela and by the end of the study period in Pranto. Vlasblom and Bolier (1971) described lower viability of *E. marinus* eggs in the presence of low salinities, which might explain the decrease in the percentage of ovigerous females in Fontela, since this station presented very low salinity values during the winter. A similar situation occurred in Pranto (Fig. 11P), but the effects on the percentage of ovigerous females were only observed by the end of the winter. Maranhão *et al.*, (2001) also observed a similar pattern by the end of winter, with a very

low percentage of ovigerous females in Pranto and Fontela associated with low salinity values for the referred sampling stations. Marques and Nogueira, (1991) observed, likewise, a period of low sexual activity allied with low salinities. These authors also obtained a positive correlation between salinity and percentage of ovigerous females and between temperature and percentage of juveniles, suggesting that temperature and salinity are the variables that have a stronger influence over sexual activity and consequently, recruitment.

This reinforces the notion that in the Mondego estuary, considered as the southern limit of its distribution, *E. marinus* reproduces throughout the year with a multivoltine cycle. A laboratory study by Maranhão and Marques (2003) also concluded that ovigerous females of *E. marinus* (from the Mondego estuary) appeared in the population 8-12 weeks after development and reproduced at least three times during their life cycle. Latitude seems to play an important role in the determination of the reproductive cycle in other populations of this amphipod, which can also be univoltine (temperate cold to sub-polar climates) (Skadsheim, 1982) or bivoltine (temperate maritime climates) (Pinkster and Broodbaker, 1980). These intraspecific variations seem to be a common feature in amphipod populations (e.g. Covi and Kneib, 1995; Moore and Wong, 1996; Costa and Costa, 1999; Pardal *et al.*, 2000)

The previous work by Marques and Nogueira (1991) was able to identify cohorts within the *E. marinus* populations of the Mondego estuary, due to the occurrence of discontinuous recruitment. However, and like Maranhão *et al.* (2001), the data obtained in this study did not allow the recognition of cohorts, making it impossible to determine growth rates and production from field data. Thus, production was estimated through the method of the sum of size frequencies, described the methods chapter (Section 2.2.3.1).

Sex-ratio presented considerable oscillations in the studied populations throughout the year, still, a predominance of male phenotypes was observed. In Pranto and Armazéns males were almost always dominant, except during autumn and partially in winter (Fig. 12P; 12A), while in Fontela females were mainly dominant from mid-summer to early autumn. This male predominance was also reported by Maranhão and co-workers (2001) but not by Marques and Nogueira (1991), whose work reported a female prevalence which seems to be the most common occurrence in *E. marinus* populations (Skadsheim, 1982) and other amphipod species (e.g. Covi and Kneib, 1995, Beare and Moore, 1996; Persson, 1999; Pardal *et al.*, 2000). Statistical analysis found no correlation between sex-ratio and any of the monitored environmental variables, opposing the positive correlations with temperature and green macroalgae biomass observed by Maranhão *et al.*, (2001) and the positive correlation with dissolved oxygen and negative with nitrate concentration obtained by Marques and Nogueira (1991). However, other variables that were not taken into account in this work, such as growth, maturation, longevity and predation rates, might affect sex-ratio (Beare and Moore, 1996). Watt (1994), demonstrated that photoperiod influenced the sex-ratio in *Gammarus duebeni*, with male predominance in longer days (>13-14h) and female predominance in shorter days (<13-14h). Food quality and availability in populations of *Uhlorchestia spartinophila* (Kneib, 1997), and microsporidean parasitism and intersexuality in other *E. marinus* populations (Ford *et al.*, 2006; Martins *et al.*, 2009) might also be important variables that affect sex-ratio. Nevertheless, it is important to remember that immature females are included in sex-ratio estimations, therefore the announced ratios do not represent the real operational ratios of reproductively fit individuals (Beare and Moore, 1996).

4.3 Production

Production estimates showed spatial variations with Armazéns having the highest annual production (6.35g AFDW.m^{-2}.y^{-1}), closely followed by Fontela (5.78g AFDW.m^{-2}.y^{-1}). These two sampling stations also presented higher average annual biomasses and very similar P/$\overline{B}$ ratios. Moreover, these sampling locations exhibited higher densities and biomass values, which contributed to a higher production. The population of Pranto, on the other hand, displayed the lowest values of annual production (2.11g AFDW.m^{-2}.y^{-1}), average annual biomass and P/$\overline{B}$ ratios, which were related to lower densities and biomasses.

Considering *Echinogammarus marinus* production from previous studies, the present values (2.11 – 6.35g AFDW.m^{-2}.y^{-1}) are higher than those observed in the study by Maranhão and co-workers (2001) (1.74 – 5.38g AFDW.m^{-2}.y^{-1}) but still far from the ones registered by Marques and Nogueira (1991) (6.36 – 8.8g AFDW.m^{-2}.y^{-1}). This is probably related with the densities observed for each decade, since the current values are higher than the ones observed in the 90's study but lower than the ones detected in the 80's, thus reinforcing the general notion that the system is recovering from the multiple impacts suffered in the last twenty years. P/$\overline{B}$ ratios (5.15 – 8.82) were also higher than the ones reported in the previous studies (6.08 – 6.31 and 1.14 – 6.36, in Marques and Nogueira, 1991 and Maranhão *et al*., 2001, respectively), mainly due to a decrease in biomass. Occurrence of higher densities but smaller individuals might be related to the population's strategy regarding the current environmental conditions of the Mondego estuary. These findings are consistent with an opportunistic **r** adaptive strategy in *E. marinus* populations (Marques and Nogueira, 1991), which is the most frequent pattern in epifaunal species, especially in systems exposed to higher environmental pressures (Van Dolah, 1980), such as the Mondego estuary.

CHAPTER 5

CONCLUSIONS

5. CONCLUSIONS

Results indicate that density, biomass and productivity exhibited differences within the three sampled *E. marinus* populations and throughout the study period. In addition, salinity and ammonia appear to be the most relevant environmental factors affecting *E. marinus* distribution.

Echinogammarus marinus density, biomass and productivity values were considerably higher than the study carried out during the 90's by Maranhão *et al.*, (2001) but they did not reach the values reported by Marques and Nogueira (1991) during the 80's. This might indicate that the Mondego estuary is still recovering from previous anthropogenic pressures and, considering the present impacts, the mitigation measures seemed to have been successful.

In the present study, the accentuated decrease detected in the density of the three populations throughout time may have been related with the duration of the study. In fact, samples obtained posteriorly indicate that the three populations of *E. marinus* from the Mondego estuary have recovered from the gradual density decrease, observed from April 2009 to March 2010 (personal observation). Thus, it is possible to conclude that a one-year period for this type of study is clearly insufficient, since seasonal patterns for density variation may not be fully established.

Contrary to a previous study by Pastorinho and co-workers (2009), intersexuality was considered insignificant (< 0.1%) for the populations of *Echinogammarus marinus* in the Mondego estuary (the southern limit for this specie's distribution).

In conclusion, this study helped to reinforce the general idea of estuaries as highly productive systems, with amphipods having a crucial role in the trophic exchanges of those systems. *Echinogammarus marinus* proved to be a key species in the

Mondego estuary, emphasizing the necessity for more audacious studies that can fully comprehend this amphipod's contribution to the trophic chains.

CHAPTER 6

REFERENCES

6. REFERENCES

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